B7 CONT.

These amendments are made without prejudice and are not to be construed as abandonment of the previously claimed subject matter or agreement with the Examiner's position. In accordance with the requirements of 37 C.F.R. § 1.121, a marked up version showing the changes to the claims, is attached herewith as Appendix A. For the Examiner's convenience, a complete claim set of the currently pending claims is also submitted herewith as Appendix B.

REMARKS

Status of the Claims.

Claims 1, 2, and 5-21 are pending and under consideration with entry of this amendment, no claims being cancelled and no claims being added herein. The specification and claims 2, 5, 6, and 11 are amended herein. These amendments introduce no new matter. The amendments to the specification are made to correct grammatical errors. Claim 2 is amended to correct claim dependency. Claims 5 and 11 are amended to make grammatical clarifications. Claim 6 is amended to correct claim dependency and to indicate that alteration in release of the recited proteins that the cell is characteristic of a trophoblast in an abnormal placental interface. Support is replete throughout the specification (*e.g.*, page 11, lines 29-31).

Claims 1, 2, and 5-21 were rejected under 35 U.S.C. §112, first paragraph, and second paragraph. Applicants respectfully traverse.

Election/Restriction.

Pursuant to a restriction requirement made final, Applicants note that claims 3, 4, and 22 are withdrawn from consideration pursuant to 37 C.F.R. §1.142(b) as being drawn to a non-elected species. Species A is presently under consideration.

Applicants recognize that, per MPEP §809.02(c), to the extent all species fall within the limitations of a generic claim ultimately determined to be patentable, the non-elected species will no longer be deemed to be withdrawn and claims to the additional non-elected species will be considered by the Examiner.

35 U.S.C. §112, Second Paragraph.

Claims 5-22 were rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite for the reasons discussed below.

A) Methods of culturing cells.

Claims 5 and 6 were rejected as allegedly indefinite because the Examiner alleged that claim 5 is darn to a method of growing a cell and is not limited to any particular protein, while dependent claim 6 recites measuring the release of the protein. The Examiner alleged that claim 6 appears to be a diagnostic method, but it was unclear what is being diagnosed.

Applicants have amended claim 6 herein, to clarify that the recited changes in protein secretion indicate that the cell is characteristic of a trophoblast in an abnormal placental interface. In other words, as taught in the specification:

> It was a discovery of the present invention that trophoblast cells cultured under **hypoxic conditions** attain a morphology, antigenic phenotype, and activity that appears identical to that observed in trophoblasts of an abnormal maternal-placental interface characteristic of various diseases of pregnancy such as threatened abortion, high intrauterine growth retardation, gestational trophoblast diseases including molar pregnancy, choriocarcinoma, placental site tumors, ectopic pregnancy, proteinuria, pregnancy induced hypertension and preeclampsia. [emphasis added] (page 3, lines 12-21)

Particularly, when read in light of the specification, claim 5 and amended claim 6 clearly identify what is being "diagnosed". These claims are not indefinite and the rejection of these claims under 35 U.S.C. §112, second paragraph, should be withdrawn.

B) The term "release".

Claims 5-22 were rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite in the use of the term "release". According to the Examiner, it is unclear what property is referred to as "release". Applicants respectfully traverse.

The Examiner is reminded that a claim is definite if:

> **[R]ead in light of the specification** [it] reasonably apprise[s] those skilled in the art both of the utilization and scope of the invention, and if the language is as precise as the subject matter permits. [emphasis added] *Hybritech Inc. v Monoclonal Antibodies, Inc.,* 802 F.2d 1367, 1385, 231 USPQ 81 (Fed. Cir. 1986) cert. denied 480 U.S. 947 (1987).

In the instant case, the specification expressly states:

> The terms "expression" or **"release"** of a protein when used herein in reference to a protein whose expression or release is altered under hypoxic conditions are used to indicate that alterations in detectable protein level are due to alterations in the physiological activity of the cell or tissue and not to attribute a particular mechanism to the observed alteration in detectable protein level. Thus the phrases in "increase in expression" or "increase in release" of a protein are used to indicate that **some action of the subject cell or tissue results in an increase in the detected levels of that protein, either released to the environment (e.g., culture medium) or detected in a lysate**. The increase can be due, for example, to increased expression of a gene encoding that protein, to defective expression of a native protein resulting in the detected protein "fragment", changes in uptake of the protein, changes in active secretion of thel protein or changes in net release of the protein.

The Examiner is simply incorrect in his statement that "it is not clear if this means release of the protein from the nucleus, from the cell into an extracellular space, ro release of bindign of the protein to a ligand." To the contrary, as indicated above, the specification expressly states that **"increase in release" of a protein are used to indicate that some action of the subject cell or tissue results in an increase in the detected levels of that protein, either released to the environment (e.g., culture medium) or detected in a lysate**.

The use of the term "release" simply does not render the claims indefinite and the rejection of claims 5-22 on this ground should be withdrawn.

C) Recitation of purposes.

The Examiner rejected claims 5-22 as allegedly "vague in the recitation of purposes such as 'abnormal placental function' for example "preeclampsia," "invasiveness of trophoblasts, determining if proteins are present in 'metastatic cells'" (*see* Office Action, page 2, last two lines). The Examiner further stated that the claims are silent on just how one carries out these complex purposes and further argued that the claims are unclear on what specific end product is to be measured and what that end product indicates. Applicants respectfully traverse.

The term "abnormal placental function" is recited in claims 6, 11, and 12. Claim 6 recites ". . . where alteration in release of the above-identified proteins as described indicates that said cell is characteristic of a trophoblast in an abnormal placental". Claim 11 recites " A method for detecting an abnormal placental function. . . ", and claim 12 recites " said abnormal placental function is a symptom of a disease of pregnancy selected from the group consisting of . . . ".

The specification expressly states:

> In contrast, anchoring villi contain cytotrophoblast stem cells that enter both differentiation pathways. In much of the anchoring villus, cytotrophoblasts fuse to form a syncytium. However, at selected sites, cytotrophoblasts break through the syncytium and form multilayered columns of nonpolarized cells. Anchoring villi physically connect the embryo to the uterine wall via these cell columns, and give rise to the most highly invasive and migratory cytotrophoblasts. These **invasive trophoblasts** (also known as intermediate trophoblasts, cytotrophoblasts, or x-cells) invade uterine blood vessels.
>
> * * *
>
> A large body of evidence indicates that preeclampsia, and other diseases of pregnancy, are associated with **highly characteristic abnormalities in placental development (referred to herein has an "abnormal maternal-placental interface") such that the placenta is only superficially connected to the uterus. Cytotrophoblast invasion is shallow and does not proceed beyond the decidual portions of the spiral arteries.** (Redman, *New Engl. J. Med.* 323: 478 (1990); Brosens *et al. Obster, Gynecol. Annu.* 1: 177 (1972) Gerretsen *et al., Brit. J. Obstet. Gynecol.*, 88: 876 (1981); Moodley and Ramsaroop, *S. Afr. Med. J.*, 75: 376 (1989)). In addition, not as many vessels show evidence of trophoblast invasion (Khong *et al. Br. J. Obstet. Gynecol.*, 93: 1049 (1986)).
>
> * * *
>
> These morphological differences are a dramatic contrast to normal development (placental differentiation) in which, as explained above, the trophoblasts, detach from their basement membranae, aggregate, and invade much of the uterus and its arterial system thereby forming an intimate connection (the maternal-placental interface) between the mother and the fetus. As used herein, **the term "abnormal placental function" refers to the physiological consequences of this abnormal placental development**. [emphasis added] (page 10, line 18 to page 11, line 9)

The terms abnormal placental function is thus expressly defined in the specification in, *e.g.* in terms of "highly characteristic abnormalities" in placental development. The term is one well known to those of skill in the art. The language clearly communicates to one of skill in the art the metes and bounds of the invention and is as precise as the subject matter permits.

The term "preeclampsia" is recited in claims 12, 13, and 22. In these claims preeclampsia is a disease state of which an abnormal placental function is a symptom. The term "preeclampsia" is well known to those of skill in the art. Again, the language clearly communicates to one of skill in the art the metes and bounds of the invention and is as precise as the subject matter permits.

The term "metastatic cells" is recited in claim 19. Again, the term "metastatic cells" is well known to those of skill in the art. The language clearly communicates to one of skill in the art the metes and bounds of the invention and is as precise as the subject matter permits.

The phrase "invasiveness of trophoblasts" is recited in claim 15. As indicated above, invasive trophoblasts are well known to those of skill in the art:

> However, at selected sites, cytotrophoblasts break through the syncytium and form multilayered columns of nonpolarized cells. Anchoring villi physically connect the embryo to the uterine wall via these cell columns, and give rise to the most highly invasive and migratory cytotrophoblasts. These **invasive trophoblasts** (also known as intermediate trophoblasts, cytotrophoblasts, or x-cells) invade uterine blood vessels. [emphasis added) (page 10, lines 18-24)

Again, the language clearly communicates to one of skill in the art the metes and bounds of the invention and is as precise as the subject matter permits.

With respect to the Examiner's comment that the claims are unclear on what specific end product is to be measured and what that end product indicates, Applicants submit that the Examiner is simply incorrect.

The claims are completely explicit about what end product is to be measured (*one of* released proteins recited in the claim and identified by molecular weight, PI, and in certain instances, sequence.) In addition the claims are completely clear about what that end product indicates. Thhus, for example, claim 11 indicates that the end product indicates abnormal placental function, which is a symptom of "a disease of pregnancy selected from the group consisting of threatened abortion, intrauterine growth retardation, gestational trophoblast diseases including molar pregnancy, choriocarcinoma, placental site tumors, ectopic pregnancy, proteinuria, pregnancy induced hypertension and preeclampsia." (*see, e.g.,* claim 12).

In view of the foregoing, Applicants submit that claims 5-22 meet the requirements of 35 U.S.C. §112, second paragraph, and the rejection of these claims as allegedly indefinite should be withdrawn.

35 U.S.C. §112, First Paragraph.

Claims 1, 2, and 5-21 were rejected under 35 U.S.C. §112, first paragraph, as allegedly not enabled. In particular, the Examiner alleged that claims 5-21 are drawn to a myriad of uses for protein A. The Examiner alleged that claim 5, drawn to a method of culturing human fetal trophoblast

cells in less than about 20% oxygen atmosphere is not even limited to the use of any single protein. He further alleged that claims 6-22 read on any use of the protein to diagnose or screen for any disease.

The Examiner also allegedly that the specification discloses that the field is unpredicatable and alleges that the specification does not show any nexus between pregnancy-specific beta-1-glycoprotein and protein A the subject of the claims. Applicants respectfully traverse.

Applicants first note that the Examiner has failed specifically indicate why he believes claims 1 and 2 are not enabled. Applicants also note that only claims 5 and 6 are directed to culture methods, not claims 5-22 as alleged by the Examiner. Nevertheless, Applicants will address the rejection with respect to all pending claims.

The Examiner is reminded that to be enabling under §112, first paragraph, a patent must contain a description that enables one skilled in the art to make and use the claimed invention. **That some experimentation is necessary does not constitute a lack of enablement**; the amount of experimentation, however, must not be unduly extensive.

Whether undue experimentation is required by one skilled in the art is typically determined by reference to eight factors considered relevant to the inquiry: (1) quantity of experimentation necessary; (2) amount of guidance presented; (3) presence of working examples; (4) nature of the invention; (5) state of the prior art; (6) relative skill of those in the art; (7) predictability of the art; and (8) breadth of the claims. *In re Wands,* 8 USPQ2d 1400 (Fed. Cir. 1988) *citing Ex parte Forman Inc.,* 230 USPQ 546 (BPAI 1986). In the instant case, Applicants explain below that the practice of the pending claims does not require undue experimentation.

A) Culture methods -- claims 5-6.

The culture methods of claims 5 and 6 do not require undue experimentation. Claim 5 is directed to "a method of culturing human fetal trophoblast cells or chorionic villi under hypoxic conditions, said method comprising **[the step of]** culturing the trophoblast cells or chorionic villi under an atmosphere comprising less than about 20% oxygen."

Culture methods are well known to those of skill in the art. It simply **does not** require undue experimentation to culture cells under hypoxic conditions. Dependent claim 6 adds the detection of an increase or decrease in particular proteins where such increase or decrease provides an indicaton of the state of the cultured cells, and in particular, indicates that the cell "cell is characteristic of a

trophoblast in an abnormal placental interface" (*e.g.* the culture system provides a good model of an abnormal placental interface).

The Examples provided illustrate culturing of cells under such conditions and the detection and isolation of the recited proteins (*e.g.* protein A). Separation and isolation of the proteins via two-dimensional electrophoresis is illustrated (*see, e.g.* Examples and Figure 1 and 2).

The specification thus provides working examples (Wands Factor 3) and detailed protocols for the claimed method. Accordingly, in view of this considerable guidance (Wands Factor 1), relatively little, or no experimentation is necessary (Wands Factor 1). The nature of the invention (Wands Factor 4) is relatively straightforward; a culture method. The prior art (Wands Factor 5), cell culture methods, is well developed. In view of the teaching provided in the specification, it is highly predicatable (Wands Factor 7) that cultured trophoblasts will achieve a phenotype as described in the specification. The skill in the art (Wands Factor 6) is high, typically Ph.D., and the claims are relatively narrow (Wands Factor 8) being drawn simply to a culture method.

Thus, when analyzed in light of In re Wands, practice of claims 5 and 6 does not require undue experimentation and the rejection of these claims under 35 U.S.C. §112, second paragraph, should be withdrawn.

B) Protein claims -- claims 1-2.

It does not require undue experimentation to make and use the proteins of claims 1 and 2. The specification expressly teaches how to isolate the claimed proteins and provides a detailed example illustrating their isolation, *e.g.* via two-dimensional electrophoresis.

Moreover the Examiner's assertion that the specification does not show any nexus between measurements of protein A and any practical use are simply unfounded. The specification expressly states:

> In particular, it is a discovery of the present invention that **hypoxic trophoblasts express a gross morphology, a histology, an antigenic phenotype and a loss of invasiveness that is identical to that found in cells characteristic of the abnormal maternal-placental interface characteristic of a number of diseases of pregnancy.** Such diseases include, but are not limited to, threatened abortion, high intrauterine growth retardation, gestational trophoblast diseases including molar pregnancy, choriocarcinoma, placental site tumors, ectopic pregnancy, proteinuria, pregnancy induced hypertension and preeclampsia. [emphasis added] (page 8, line 28 to page 9, line 3).

* * *

> **These proteins provide useful markers for the early diagnosis of abnormal placental function and thus of diseases, such as preeclampsia, characteristic of abnormal placental function.** As it is believed that these proteins are responsible for various complications of pregnancy including (1) an alteration in vascular reactivity associated with a hypersensitivity to infused angiotensin; (2) a decrease in production of prostacyclin with an associated increase in production of thromboxanes; (3) a decrease in renal hemodynamics, due at least in part to glomerular endotheliosis; (4) a widespread endothelial disorder, resulting in the loss of albumin from the intravascular space; and (5) an inherent immunologic misadaptation during placentation, resulting in incomplete trophoblast invasion of the spiral arterioles pathophysiological abnormalities. **Detection of expression levels of these proteins provides a screening system for possible therapeutic agents that might mitigate these and other adverse effects of abnormal placental differentiation.** [emphasis added] (page 9, lines 9-21)

The specification thus expressly teaches that the proteins are useful markers for the physiological state of trophoblasts and provide and indication of the state of placental formation during gestation. As indicated above, the specification also teaches that " provides a screening system for possible therapeutic agents that might mitigate these and other adverse effects of abnormal placental differentiation." Contrary to the Examiner's assertion, Applicants have established a nexus between protein A expression and an abnormal placental interface. Accordingly there are clear uses for the claimed proteins.

When considered in terms of the Wands factors, as explained above, the specification clearly teaches and illustrates the isolation of protein A. The specification thus provided guidance and working examples (Wands Factors 2 and 3) and little experimentation (Wands Factor 1) is necessary. The state of the prior art (Wands Factor 5) and the nature of the invention (Wands Factor 4), protein isolation is well developed. In view of the teachings provided in the specification the art is quite predictable (Wands Factor 8), and the skill of those in the art is high (Wands Factor 7). Finally, claims 1 and 2 are relatively narrow (Wands Factor 8) being drawn to a particular protein.

When analyzed in light of *In re Wands*, isolation of the protein(s) of claims 1 and 2 does not require undue experimentation and the rejection of these claims under 35 U.S.C. §112, second paragraph, should be withdrawn.

C) Diagnostic/screening methods -- claims 7-21.

Undue experimentation is not required to practice the screening methods of claim 7-21. As explained above, it was a discovery of this invention that the recited changes in protein levels are indicative of an abnormal placental placental interface:

> It was a discovery of the present invention that trophoblast cells cultured under hypoxic conditions attain a morphology, antigenic phenotype, and activity that appears **identical to that observed in trophoblasts of an abnormal maternal-placental interface** characteristic of various diseases of pregnancy such as threatened abortion, high intrauterine growth retardation, gestational trophoblast diseases including molar pregnancy, choriocarcinoma, placental site tumors, ectopic pregnancy, proteinuria, pregnancy induced hypertension and preeclampsia. [emphasis added] (page 3, lines 12-21).

> These proteins provide **useful markers for the early diagnosis** of abnormal placental function and thus of diseases, such as preeclampsia, characteristic of abnormal placental function. As it is believed that these proteins are responsible for various complications of pregnancy including (1) an alteration in vascular reactivity associated with a hypersensitivity to infused angiotensin; (2) a decrease in production of prostacyclin with an associated increase in production of thromboxanes; (3) a decrease in renal hemodynamics, due at least in part to glomerular endotheliosis; (4) a widespread endothelial disorder, resulting in the loss of albumin from the intravascular space; and (5) an inherent immunologic misadaptation during placentation, resulting in incomplete trophoblast invasion of the spiral arterioles pathophysiological abnormalities. [emphasis added] (page 9, line 9-19).

The Examiner's comments regarding the unpredictability of the art and requiring a nexus between protein A and "pregnancy -specific beta-1-glycoprotein") (*see* Office Action, page 4) do not support his assertion that undue experimentation is required to practice the invention.

The art **is not** unpredictable with respect to the present invention because Applicants have established the relationship between the recited protein expression and the occurrence of the abnormal placental interface. In view of the teaching provide in the specification, with respect to the diagnostic value of the recited proteins, the art is not unpredicatable. As stated in the specification:

> A large body of evidence indicates that preeclampsia, and other diseases of pregnancy, are associated with **highly characteristic abnormalities** in placental development (referred to herein has an "abnormal maternal-placental interface") such that the placenta is only superficially connected to the uterus. Cytotrophoblast invasion is shallow and does not proceed beyond the decidual portions of the spiral arteries. (Redman, *New Engl. J. Med.* 323: 478 (1990);

> Brosens *et al. Obster, Gynecol. Annu.* 1: 177 (1972) Gerretsen *et al., Brit. J. Obstet. Gynecol.*, 88: 876 (1981); Moodley and Ramsaroop, *S. Afr. Med. J.*, 75: 376 (1989)). In addition, not as many vessels show evidence of trophoblast invasion (Khong *et al. Br. J. Obstet. Gynecol.*, 93: 1049 (1986)). [emphasis added] (page 10, line 27 to page 11, line 3).

The specification thus teaches that the developmental abnormalities with which the present invention is concerned are **"highly characteristic"** (*i.e.* reproducible). **Applicants have demonstrated the relationship between the variations of protein A release and the abnormal placental function. There is thus no necessity to demonstrate a relationship between protein A and "pregnancy -specific beta-1-glycoprotein**. Moreover, the Examiner has provided no objective evidence to refute Applicants' assertion regarding the predictive/diagnostic value of the recited proteins. Lacking any such evidence, the Examiner has failed to make his *prima facie* case under 35 U.S.C. §112, first paragraph.

Moreover, as explained above, in view of the guidance (Wands Factor 2), working examples (Wands Factor 3), relatively little experimentation (Wands Factor 1) is necessary. The nature of the invention (Wands Factor 4) is well established (developmental indicators). Similarly the prior art is well developed (Wands Factor 5). The level of skill in the field is high (Wands Factor 6). In view of the highly characteristic nature of the abnormal plancental interface discussed in the specification, the art is predictable (Wands Factor 7). Moreover, the claims are relatively narrow (Wands Factor 8), being drawn to diagnostic/screening methods.

When analyzed in light of *In re Wands*, the methods of claims 7-21 do not require undue experimentation and the rejection of these claims under 35 U.S.C. §112, second paragraph, should be withdrawn.

Change in Correspondence Address.

A Revocation and Substitute Power of Attorney incorporating a change in correspondence address was filed on April 30, 2001 and a copy of this document is enclosed herewith. In accordance with the instructions provided therein, please direct all future correspondence regarding the subject application to CUSTOMER NUMBER 22798, that is:

LAW OFFICES OF JONATHAN ALAN QUINE
P.O. BOX 458
Alameda, CA 94501
Tel: (510) 337-7871 / Fax: (510) 337-7877

In view of the foregoing, Applicants believes all claims now pending in this application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested.

If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (510) 337-7871.

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Respectfully submitted,

Tom Hunter
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APPENDIX A

VERSION WITH MARKINGS TO SHOW CHANGES MADE IN 09/101,283 WITH ENTRY OF THIS AMENDMENT

In the specification:

Page 6, line 23 to page 7, line 4:

The term **[A]**"invasiveness"**[@]**, as used herein refers to the ability of a cell to penetrate an extracellular matrix. Methods of measuring invasiveness are well known to those of skill in the art (see, *e.g.*, Librach *et al. J. Cell. Biol.*, 113: 437-449 (1991)). Similarly, an " invasive cell type" or an **[A]**"invasive cell"**[@]** refer to a cell capable of penetrating a tissue other than the tissue from which the cell originates. Invasive cells include, but are not limited to trophoblast and malignant cancer cells.

The term **[A]**"protein"**[@]**, when used herein refers to a chain of amino acids whose α carbons are linked through peptide bonds. Proteins include native proteins *in vivo* or isolated native proteins. Proteins also include chemically or recombinantly synthesized proteins. In addition, it is to be understood that the term proteins, as used herein includes the protein product as translated from an mRNA molecule as well as the protein products as subsequently modified. Thus proteins also include modified proteins such as glycoproteins, lipopoproteins and the like.

Page 7, lines 19-29:

The terms **[A]**"expression"**[@]** or **[A]**"release"**[@]** of a protein when used herein in reference to a protein whose expression or release is altered under hypoxic conditions are used to indicate that alterations in detectable protein level are due to alterations in the physiological activity of the cell or tissue and not to attribute a particular mechanism to the observed alteration in detectable protein level. Thus the phrases in **[A]**"increase in expression"**[@]** or **[A]**"increase in release"**[@]** of a protein are used to indicate that some action of the subject cell or tissue results in an increase in the detected levels of that protein, either released to the environment (e.g., culture medium) or detected in a lysate. The increase can be due, for example, to increased expression of a gene encoding that protein, to defective expression of a native protein resulting in the detected protein **[A]**"fragment"**[@]**, changes in uptake of the protein, changes in active secretion of the protein or changes in net release of the protein.

Page 10, lines 4-16:

As used herein, the term **[A]**"trophoblasts"**[@]** includes the cytotrophoblast stem cells and lineages derived from these stem cells. The various lineages derived from cytototrophoblast stem cells are generally known to those of skill in the art. In humans, for example, two differentiation pathways exist for cytotrophoblasts, giving rise to populations that are morphologically and functionally distinct (Cross *et al. Science*, 266: 1508-1518 (1994). In the first trimester, cytotrophoblast stem cells reside in chorionic villi of two types; **[A]**"floating"**[@]** villi that do not contact the uterine wall and **[A]**"anchoring"**[@]** villi that do contact the uterine wall. Cytotrophoblasts in the floating villi exist only as polarized epithelial monolayers, anchored to a basement membranae and surrounding a stromal core containing fetal blood vessels. These cytotrophoblasts, which are highly proliferative in the first trimester of gestation, differentiate exclusively by fusing to form a syncytial layer that covers the villus. Floating villi, which make up the fetal compartment of the placenta, are bathed by maternal blood and perform gas and nutrient exchange functions.

Page 10, line 27 through page 11, line 9:

A large body of evidence indicates that preeclampsia, and other diseases of pregnancy, are associated with highly characteristic abnormalities in placental development (referred to herein has an **[A]**"abnormal maternal-placental interface"**[@]**) such that the placenta is only superficially connected to the uterus. Cytotrophoblast invasion is shallow and does not proceed beyond the decidual portions of the spiral arteries. (Redman, New Engl. J. Med. 323: 478 (1990); Brosens et al. Obster, Gynecol. Annu. 1: 177 (1972) Gerretsen et al., Brit. J. Obstet. Gynecol., 88: 876 (1981); Moodley and Ramsaroop, S. Afr. Med. J., 75: 376 (1989)). In addition, not as many vessels show evidence of trophoblast invasion (Khong et al. Br. J. Obstet. Gynecol., 93: 1049 (1986)).

These morphological differences are a dramatic contrast to normal development (placental differentiation) in which, as explained above, the trophoblasts, detach from their basement membranae, aggregate, and invade much of the uterus and its arterial system thereby forming an intimate connection (the maternal-placental interface) between the mother and the fetus. As used herein, the term **[A]**"abnormal placental function"**[@]** refers to the physiological consequences of this abnormal placental development.

In the claims:

2. (Once amended) A protein of claim [**(a)**]**1**, wherein the protein is selected from the group consisting of:

(a) Protein A having a molecular weight of about 21 kDa and a pI of 6.0 wherein the release of said protein, under hypoxic conditions, is increased;

(b) Protein B having a molecular weight of about 22 kDa and a pI of 7.0 wherein the release of said protein, under hypoxic conditions, is increased;

(c) Protein C having a molecular weight of about 23 kDa and a pI of 7.5 wherein the release of said protein, under hypoxic conditions, is increased;

(d) Protein D having a molecular weight of about 55 kDa and a pI of 8.5 wherein the release of said protein, under hypoxic conditions, is increased; and,

(e) Protein E having a molecular weight of about 62 kDa and a pI of 5.5 wherein the release of said protein, under hypoxic conditions, is increased.

5. (Once amended) A method of culturing human fetal trophoblast cells or chorionic villi under hypoxic conditions, said method comprising **[the step of]** culturing the trophoblast cells or chorionic villi under an atmosphere comprising less than about 20% oxygen.

6. (Once amended) A method of claim [**4**]**5**, wherein the method further comprises measuring the release of a protein selected from the group consisting of:

(a) Protein A having a molecular weight of about 21 kDa and a pI of 6.0 wherein the release of said protein, under hypoxic conditions, is increased;

(b) Protein B having a molecular weight of about 22 kDa and a pI of 7.0 wherein the release of said protein, under hypoxic conditions, is increased;

(c) Protein C having a molecular weight of about 23 kDa and a pI of 7.5 wherein the release of said protein, under hypoxic conditions, is increased;

(d) Protein D having a molecular weight of about 55 kDa and a pI of 8.5 wherein the release of said protein, under hypoxic conditions, is increased;

(e) Protein E having a molecular weight of about 62 kDa and a pI of 5.5 wherein the release of said protein, under hypoxic conditions, is increased;

(f) Protein F having a molecular weight of about 40 kDa and a pI of 4.5 wherein the release said protein, under hypoxic conditions, is decreased;

(g) Protein G having a molecular weight of about 67 kDa and a pI of 6.5 wherein the release of said protein, under hypoxic conditions, is decreased; and

(h) Protein H having a molecular weight of about 75 kDa and a pI of 9.0 wherein the release of said protein, under hypoxic conditions, is decreased;

(i) A protein of spot number 2 comprising an amino acid sequence selected from the group consisting of sequence 1, and sequence 2 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is decreased;

(j) A protein of spot number 3 comprising an amino acid sequences selected from the group consisting of sequence 3, sequence 4, sequence 5, and sequence 6 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is decreased;

(k) A protein of spot number 5 comprising amino acid sequence number 7 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is increased;

(l) A protein of spot number 7 comprising amino acid sequence number 8 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is increased;

(m) A protein of spot number 10 comprising an amino acid sequence selected from the group consisting of sequence 12, and sequence 13 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is increased;

(n) A protein of spot number 11 comprising an amino acid sequence selected from the group consisting of sequence 14, sequence 15, sequence 16, sequence 17, and sequence 18 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is decreased; and

(o) A protein of spot number 20 comprising an amino acid sequence selected from the group consisting of sequence 21, and sequence 22 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is increased; and

(p) A human apolipoprotein A-1 wherein the release of said protein, under hypoxic conditions, is increased

where alteration in release of the proteins as described above indicates that said cell is characteristic of a trophoblast in an abnormal placental interface.

11. A method for detecting an abnormal placental function **said method comprising :[by]**

[analysing]analyzing a biological sample from a pregnant mammal for abnormal release of a protein**, wherein said abnormal release is** selected from the group consisting of:

(a) Protein A having a molecular weight of about 21 kDa and a pI of 6.0 wherein the release of said protein, under hypoxic conditions, is increased;

(b) Protein B having a molecular weight of about 22 kDa and a pI of 7.0 wherein the release of said protein, under hypoxic conditions, is increased;

(c) Protein C having a molecular weight of about 23 kDa and a pI of 7.5 wherein the release of said protein, under hypoxic conditions, is increased;

(d) Protein D having a molecular weight of about 55 kDa and a pI of 8.5 wherein the release of said protein, under hypoxic conditions, is increased;

(e) Protein E having a molecular weight of about 62 kDa and a pI of 5.5 wherein the release of said protein, under hypoxic conditions, is increased;

(f) Protein F having a molecular weight of about 40 kDa and a pI of 4.5 wherein the release of said protein, under hypoxic conditions, is decreased;

(g) Protein G having a molecular weight of about 67 kDa and a pI of 6.5 wherein the release of said protein, under hypoxic conditions, is decreased; and,

(h) Protein H having a molecular weight of about 75 kDa and a pI of 9.0 wherein the release of said protein, under hypoxic conditions, is decreased;

(i) A protein of spot number 2 comprising an amino acid sequence selected from the group consisting of sequence 1, and sequence 2 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is decreased;

(j) A protein of spot number 3 comprising an amino acid sequences selected from the group consisting of sequence 3, sequence 4, sequence 5, and sequence 6 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is decreased;

(k) A protein of spot number 5 comprising amino acid sequence number 7 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is increased;

(l) A protein of spot number 7 comprising amino acid sequence number 8 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is increased;

(m) A protein of spot number 10 comprising an amino acid sequence selected from the group consisting of sequence 12, and sequence 13 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is increased;

(n) A protein of spot number 11 comprising an amino acid sequence selected from the group consisting of sequence 14, sequence 15, sequence 16, sequence 17, and sequence 18 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is decreased; and

(o) A protein of spot number 20 comprising an amino acid sequence selected from the group consisting of sequence 21, and sequence 22 as shown in Table 2 and wherein the release of said protein, under hypoxic conditions, is increased; and

(p) A human apolipoprotein A-1 wherein the release of said protein, under hypoxic conditions, is increased.